Oregon Harmful Algae Bloom Surveillance (HABS) Program

Sampling Guidelines for Cyanobacterial Harmful Blooms in Recreational Waters

Public Health Division
Center for Health Protection
Research & Education Section

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These sampling guidelines represent a consensus view among experts and stakeholders involved in CyanoHABs issues either through public health protection, resource management, research, or environmental regulation. The guidelines were reviewed and approved by laboratories that specialize in cyanobacteria analysis. In any case, ***contact the laboratory prior to sampling to confirm sample collection and preservation requirements.***

Background

Cyanobacteria, also known as blue-green algae, are commonly found in many freshwater environments around the world. Some cyanobacteria species are referred to as toxigenic because they have the potential to produce toxins that can harm people, pets and wildlife.

The Oregon Public Health Division (OPHD) is working to gain a better understanding about the occurrence of cyanobacterial harmful blooms (cyanoHABs) in Oregon and their impact on human health. This work is funded is through a five-year federal grant (10/2008 – 9/2013) from the U.S. Centers for Disease Control and Prevention (CDC).

Part of the cooperative agreement with the CDC includes building the capacity of lake management agencies to monitor water bodies in a scientifically sound manner with the goal of protecting public health. The OPHD relies on these lake managers to collect environmental samples that are the basis for public health advisory decisions. The OPHD works in partnership with local lake management agencies when issuing or lifting public health advisories.

Purpose of this document

The purpose of this document is to provide guidance for lake managers who intend to monitor water bodies when potentially harmful algae blooms are detected. This guidance document encompasses all sample collection for algae identification, enumeration and toxin analysis at freshwater bodies with the objective of protecting public health, specifically with respect to general recreational uses. This guidance is not intended to encompass sample collection for environmental/ecological assessment or for community water system protection.

Sampling guidance

The goal of this program is to collect water samples that are representative of public health protection for general recreational uses. Assessing the risk posed by toxic cyanobacteria or the potential for development of cyanobacterial blooms, and linking this to effective measures for the protection of public health within available resources, is complex. Please use the following considerations when deciding to take the sample.

Visual Assessment: A Precursor to Sampling

Simple visual assessment is an important tool in recognizing cyanobacteria. A visual assessment of the bloom status of the water body, such as areas of discoloration or surface scum collection, should be used to guide sampling. Materials to help identify cyanobacteria (e.g., a field guide) provide an early-warning mechanism to help address concerns about cyanoHABs.

Steps for Visual Assessment:

- Determine if a bloom is present based on water color and the level of cyanobacteria visible on the surface and suspended throughout the water column.
- Describe the location of any areas of concentrated algae or floating mats (e.g., in and around swimming areas).
- Take photos of the areas with concentrated algae or floating mats.
- Record results of the visual assessment on a field data sheet; refer to Appendix A for an example.
- Contact the OPHD if a bloom is present.

Sample collection: Where, when and how

Monitoring should focus primarily on the protection of human health and secondarily on the health of pets and livestock. Visual observation of an algae bloom is the trigger for water sampling and is also a key consideration when determining optimal sample locations.

Where - Assessing the potential hazard at recreational water bodies can be complicated if there are numerous access points that allow people and animals to enter or move around the water. Important factors to consider when sampling are: cyanobacteria often accumulate along shoreline or as floating mats, cyanobacteria concentrations often rapidly change due to wind or other factors, and scums can generally be assumed to present the greatest risk to recreational bathers. Monitoring should include samples that represent worst-case conditions in areas in which people and animals are most likely to contact the water.

Both the location and the number of samples will depend upon the specific needs as determined by recreational use and available funding. If funding is limited, sampling should focus on near shore waters in areas where wading and swimming might occur. If resources allow, sampling far from shore may be desired in order to assess risks to water skiers and other recreational uses.

When - When you sample during the course of the day can be as important as where. Throughout the day, as temperatures warm, cyanobacteria can move closer to the surface and therefore change what you might expect to collect in a sample. Also, prevailing winds can change throughout the day which can influence where the bloom might be found as well as how well-mixed the water will be. When you are planning your sample collection activity, also keep in mind what time of day you would expect recreation to take place. Ideally, you would plan your sample during the middle of the day when it tends to be warmer and calmer.

When to Conduct Follow-up Samples - The response to each bloom is unique and will vary according to the characteristics of the particular bloom and the affected water body. Listed below are some other considerations which may drive decisions as to when to collect follow-up samples.

- *Are you conducting toxin-based monitoring (see page 6)?* If toxin-based monitoring is in progress and toxin concentrations are below guideline levels, then sample every other week. In all cases, consider the following scenarios to determine when to sample.
- *Is there a change in visual observations (e.g. decline in bloom)?* Visual observations are important when determining re-samples. It is advisable to follow the progress of the bloom, observing changes in its size, location and intensity.
- *Has there been a report of an animal death or human illness?* Any such reports are an important indication that further samples, such as toxin analysis, should be taken.
- *Does the weather forecast call for rain?* Periods of cool rainy weather can often lead to the disappearance of a bloom. On the other hand, rain may introduce nutrients that can lead to the resurgence of a bloom.
- *Is the wind or current enough to move the bloom from the initial sample location?* Weather can influence where cyanobacteria will accumulate in a given location. Wind and waves may cause blooms to form along shorelines or in protected areas; shifts in wind direction can move a bloom from one location to another.

- *Is the temperature rapidly falling?* Cyanobacteria blooms generally do not persist through the winter months due to low water temperatures.

How – The purpose of standardized sample collection methods is to balance sampling that is representative of field conditions, minimizes variability, yet meets program goals and resources. See Appendix B for a list of field equipment and step-by-step instructions for collecting water samples. These instructions produce samples that represent the risk of exposure to worst case scenarios of contacting the surface and upper layer of water while wading, swimming or skiing.

Grab or single point sampling is the most basic method used to represent a particular recreational area and/or bloom. Additional grab samples can be taken to represent other areas of concern at the waterbody. All analytical results from all samples taken at the waterbody are considered in the public health advisory process, with the result applying to the entire waterbody.

Composite sampling is a method used to minimize sample variability. Composite samples use a variety of methods to combine samples taken at a given point across time, at a given location at many depths, or at many locations at a given depth. Use the following guidelines for composite sampling for the purpose of public health protection from cyanoHABs:

- The composite sample should combine three samples taken to represent worst-case conditions. Look for areas with the most scum or thickest cyanobacteria.
- Do not combine samples taken both within a bloom and outside of the bloom in a "clean" area. This may significantly dilute the result and give a false negative.
- Do not combine samples taken from different blooms; they can have significantly different composition and toxin profiles.
- Likewise, do not combine samples taken from different recreational areas.

Safety

Cyanobacteria can produce potent neurotoxins and hepatotoxins, so exercise care when collecting water samples. In addition, cyanobacteria produce lipopolysaccharides that can irritate the skin. Wear protective clothing such as hip waders and long rubber gloves whenever necessary.
Do not ingest any amount of surface water and avoid inhalation of aerosolized (e.g., by a boat motor) surface water. Always wash hands and other exposed areas (feet, legs, etc.) with soap and water after collecting samples. Wash boots and gloves off with clean water after exposure to cyanobacteria.

The overarching precaution when sampling is personal safety. When selecting sampling locations, look for ease of access, and make sure there are no physical safety barriers.

Lugol's solution should be used to preserve cell enumeration and species identification samples. This preserving agent is commonly used for short-term (e.g. a few months, but possibly up to a year or more) storage of cyanobacteria. Check with a contract laboratory to determine how to obtain Lugol's solution.

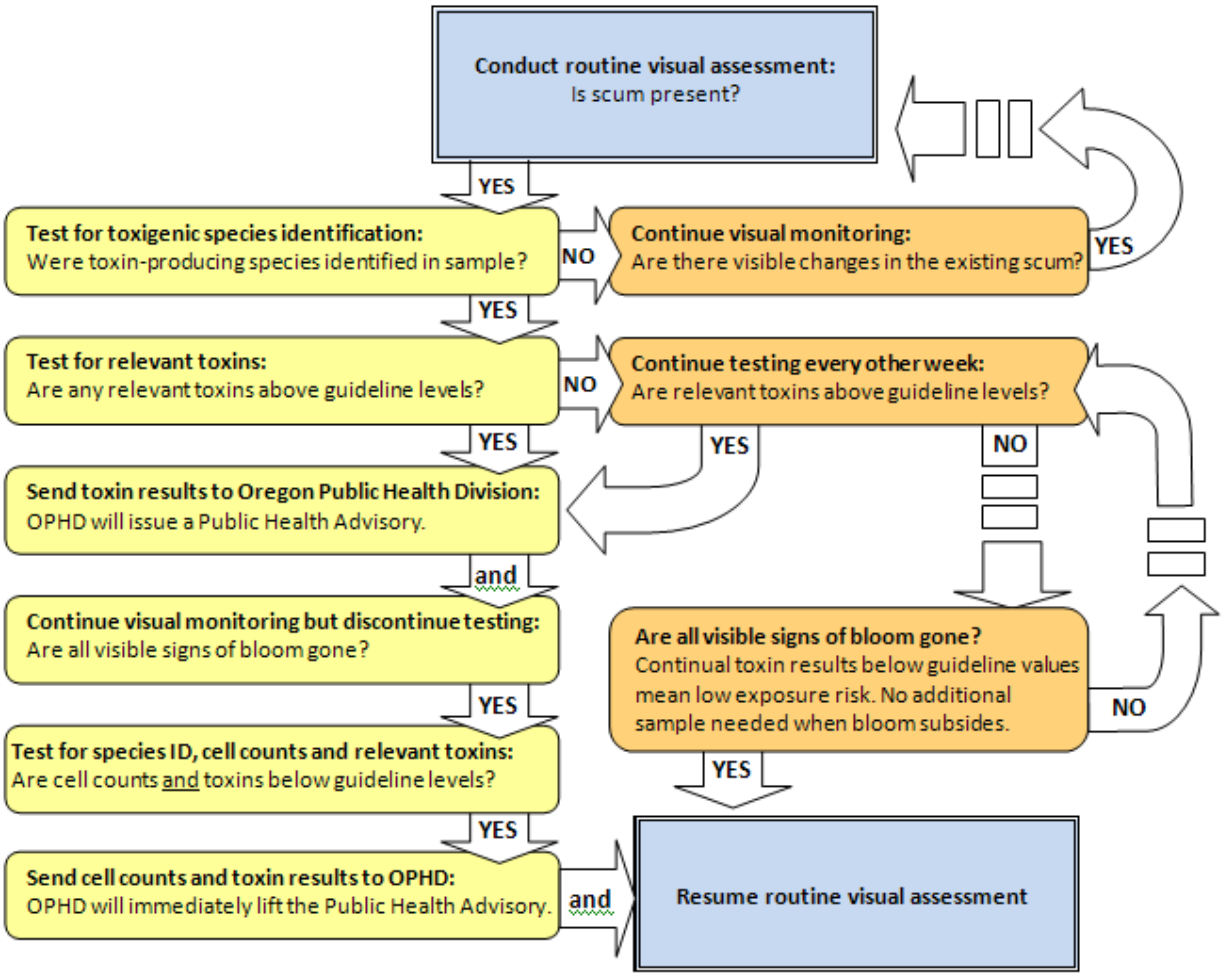
Wear gloves and eye protection when handling Lugol's. Add it to the sample(s) in a well-ventilated area (e.g., in a fume hood or outdoors).

For more information on Lugol's solution, please read the Material Safety Data Sheet (MSDS) for the hazards of this chemical.

Toxin-Based Monitoring Program: Option 4

The Public Health Advisory Guidelines document explains that a monitoring program based on toxins rather than cell counts will likely result in fewer and shorter duration public health advisories, but may have higher sampling costs. Toxin-based monitoring programs also provide the most accurate information in terms of protecting public health because toxins pose actual risk rather than the potential risk posed by the presence of cyanobacterial cells.

The recommended approach to maintaining a toxin-based monitoring program is as follows:



If Option 4 is chosen an advisory will not be issued unless toxin testing shows levels above the guideline values shown in Table 1. In the interim, the public should be notified that a bloom has been identified and toxin testing is underway or ongoing. The public should be advised to avoid water that is foamy, scummy, thick like paint, pea-green, blue-green or brownish red. Contact OPHD for signage options.

Toxin-based monitoring program sampling frequency is every other week IF there are toxin-producing species present but the toxin levels are below guideline values. When using Option 4 toxin testing, OHA recommends sampling data be available within 3 business days after the lab receives the sample. This helps us to meet the need for timely communication with the public, and demonstrates that action is being taken to protect the public health. Before hiring a lab to process

results, we recommend contacting them to discuss average turnaround time for testing to ensure conformity with this timeframe. For OHA purposes, electronic versions with official, final results will suffice. If levels are found above guideline values then an advisory is issued, advisory signage is posted and sampling can be ceased until the bloom is gone. To lift the advisory, samples must simultaneously show that cell counts and toxin levels are below guideline values.

NOTE: OPHD is interested in seeing all toxin and cell speciation data for tracking purposes only. Providing these results is not required for participation in OPHD's cyanoHABs program. However, it would help OPHD to build a statewide database on the trends in cyanoHABs in Oregon waters. OPHD will take no action on any results submitted unless results are above threshold values.

Laboratories

The number of laboratories available for species identification, cell counts, and toxin analysis is limited. Laboratories providing cyanobacterial analysis are listed in Appendix C.

The accepted method for determining cell counts is Standard Methods Section 10200E and F (also called "SM10200"). The cost for species identification and cell counts is approximately $150.

Toxin analyses are required to lift public health advisories for cyanoHABs. Cyanotoxin analyses currently available through commercial laboratories use a variety of comparable methods. These analyses may be quite costly, ranging from $150 to $350 depending on the method and equipment used. For microcystin and cylindrospermopsin determination, the ELISA method is least expensive. ELISA methods are not currently available for anatoxin-a or saxitoxin.

Note: OPHD will not accept field ready test kits for microcystin or cylindrospermopsin as a basis for lifting an advisory. However, the kits may be useful for monitoring the progress of a bloom on various water bodies throughout the season.

When testing for cyanotoxins, ensure the lab uses a method detection level that is less than the guideline values in Table 1.

Table 1. Health advisory guideline values for cyanotoxins in Oregon's recreational waters

	Anatoxin-A (µg/L)	Cylindrospermopsin (µg/L)	Saxitoxin (µg/L)	Microcystin (µg/L)
Guideline Value	20	6	100	10

It is important to test for the toxins that are potentially produced by the dominant species of cyanobacteria in a specific water body. To do this, it is necessary to identify the dominant species present (without enumeration) and consult Table 2 to determine the associated toxins.

Note: Table 2 is at the genus level, not the species level. Not all species of a given genus produce all of the toxins listed for that genus. Once the species involved in a specific bloom have been identified, OPHD recommends that water body mangers contact OPHD to determine exactly which toxins could be involved. Also note that for the purpose of public health advisory, ***Aphanizomenon flos-aquae (AFA) is excluded from calculation of combined cell counts of toxigenic species.*** *See the Public Health Advisory Guidelines for more information.*

Table 2- Toxigenic cyanobacteria (data are derived from evidence of toxin production presented in Sivonen and Jones 1999, Carey et al 2007, Funari and Testai 2008 and Voloshko 2008).

	Hepatotoxins			Neurotoxins	
	Microcystin	Nodularin	Cylindro-spermopsin	Anatoxin-a	Saxitoxin
Anabaena	+		+	+	+
Anabaenopsis	+				
Aphanizomenon (Except A. flos-aquae)			+	+	+
Arthrospira	+				
Cyanobium	+				
Cylindrospermopsis			+		+
Gloeotrichia	+				
Hapalosiphon	+				
Limnothrix	+				
Lyngba					+
Microcystis	+			+	
Nodularia		+			
Nostoc	+				
Oscillatoria	+			+	
Phormidium	+			+	
Planktothrix	+			+	+
Raphidiopsis			+	+	
Schizothrix					
Synechocystis	+				
Umezakia			+		

Shipment of Samples

Samples should be shipped the same day as collected. Ship the samples in a cooler or a cardboard box lined with plastic bags (to prevent leakage) and newspaper (for insulation).

For toxin samples, include ice bags, ice packs, or other cooling products (e.g., blue ice) to ensure the samples stay cold. If shipping algae identification, enumeration and toxins samples to the same laboratory, you may package them in the same cooler to save shipping costs.

Collection volume, methods of storing, holding times and shipping guidance is provided in Appendix B. **Prior to sample collection, discuss with the laboratory performing the analyses.**

Program Contact Information

Email: habhealth@state.or.us
Phone: (971) 673-0440 Toll free: (877) 290-6767 and press 0

Website: www.healthoregon.org/hab

Appendix A: Field Observation Sheet

Please gather the following information for each observation location, if applicable.

General Information

Observation date ___/___/_____mm/dd/yyyy Time _____AM / PM *(circle one)*

Name of water body _____ County _____

Observation location description/number _____

Observation Location Latitude _____ Longitude _____

Visual Assessment

Water clarity *(circle all that apply):* Clear Cloudy Hazy Opaque Don't know

Water color *(circle all that apply):* Green Blue-green Brown Rust Milky White Red Pink Clear Don't know

Visible bloom *(circle one)*: Yes No Don't know Visible scum *(circle one)*: Yes No Don't know

Reason for visual assessment: Monitoring Fish kill Health event response Other _____

Recreational Area Observations

Total number of people in the recreational area _____ Of those, number of people in the water _____

Total number of boats in use _____ Number of people waterskiing/boarding _____

Total number of dogs in the recreational area_____ Of those, number of dogs in the water _____

Other Observations/Comments

(e.g., draw a sketch, mark location of bloom with respect to recreational areas)

Sampler Information

Name _____ Position _____

Phone Number _____ Agency/Organization _____

Appendix B: Step-by-step Sample Collection

(*Contact the laboratory prior to sampling to confirm collection and preservation requirements*)

Sampling Equipment Checklist (confirm with laboratory)

- ☐ *Field Observation Sheet*
- ☐ *Arm-length Disposable Waterproof Gloves*
- ☐ *Hip Waders*
- ☐ *Eye Protection/Safety Glasses*
- ☐ *Waterproof Permanent Marker*
- ☐ *4 liter stainless steel bucket (if compositing)*
- ☐ *Cooler or insulated shipping box*
- ☐ *Ice Pack(s)*
- ☐ *GPS Unit (recommended)*
- ☐ *500 mL HDPE Plastic Sample Bottles*
- ☐ *Lugol's Preservative Solution*
- ☐ *Long-handled stainless steel stirring spoon (if compositing)*

Sampling Instructions

Sample Event Preparation

1. Prepare Field Observation Sheets (Appendix A). Use one sheet per sample location to describe ambient conditions. This will help to interpret results, as well as to provide sample collection contact information. Use pencil or waterproof permanent marker when completing the sheet.
2. Label each bottle with the sample location and either "Cell & Species" or "Toxins" as needed. Use pencil or waterproof permanent marker when completing the labels.

Collecting Samples - *Wear disposable gloves and any other necessary protective clothing.*

1. Select a sampling area that represents a distinct cyanoHAB and/or distinct risk of exposure (for example, a swim beach). Within that sampling area, select the location (or three locations, if compositing samples) with the highest concentration of cyanobacteria.
2. Approach (if taking the sample from the shoreline), wade or boat slowly to the sampling location. If wading or boating, approach on the downwind side and avoid agitating the water or sediment.
3. Disturb the water at the sample location for approximately ten seconds to simulate conditions created by a swimmer or wader coming into contact with the cyanobacteria.
4. Remove the cap from the sampling bottle, tilt the bottle approximately 45 degrees and allow the bottle to fill as you submerge it 3 to 6 inches below the surface.
5. Replace the cap and remove any cyanobacteria adhered to the outside of the bottle. Turn the bottle end-over-end 3-4 times to mix.
6. If compositing samples:
    a. Note that because cyanotoxins are organic compounds, the sampling equipment used to collect and composite samples should be made of fluorocarbon polymers, such as Teflon®; metals, such as stainless steel; or glass.
    b. Rinse the bucket with fresh water or with lake water relatively free from cyanobacteria.
    c. Pour the three sample bottles, representing the sampling area, into the bucket.
    d. Use a long-handled stainless steel stirring spoon to thoroughly mix the sample. Be aware that vigorous mixing may rupture (lyse) the cyanobacteria cells, making cell enumeration difficult. This is less of an issue for toxin samples since cyanobacteria cells in the sample will be lysed at a later stage of processing.

e. Pour into a rinsed sampling bottle. Replace the cap and remove any cyanobacteria adhered to the outside of the bottle. Turn the bottle end-over-end 3-4 times to mix.

Sample preservation – (*confirm with laboratory)*

A. Cell Enumeration & Species Identification Samples: *Handle preservatives only under good ventilation, wearing gloves and safety glasses.*

1. Remove the cap and pour off enough sample to leave one inch air space for mixing the Lugol's solution.
2. Add 5 mL Lugol's Solution (or another preserving agent) to the sample. Use a pipette, calibrated dropper, or medicine cup to measure the correct amount.
3. Close the cap tightly and invert the bottle 3-4 times to mix.
4. Send samples on ice overnight in an insulated box or cooler to the laboratory.

B. Toxin Samples:

1. Place the bottle(s) on ice as soon as possible. Send samples on ice overnight in an insulated box or cooler to the laboratory.

Appendix C: Qualified laboratories for cyanobacteria testing

When processing water samples for toxins as part of a toxin based montioring program, OHA recommends sampling data be available within 3 business days after receipt of a sample. This helps OHA and the waterbody managers to meet the need for timely communication with the public, and demonstrates that action is being taken to protect the public health. For OHA purposes, electronic versions with official, final results will suffice.

Aquatic Analysts
43 Telegraph Lane, Friday Harbor, WA 98250
(identification and enumeration)

Attn: Jim Sweet
phone: (503) 869-5032
jwsweet@aol.com

Aquatic Services
42184 Tweedle Lane, Seaside, OR 97138
(consulting, identification and enumeration)

Attn: Wayne W. Carmichael, PhD
phone: (937) 620-4603, (503) 755-0711
wayne.carmichael@wright.edu

Beagle Bioproducts, Inc
959 Schrock Road
Columbus, OH 43229
stephanie.smith@beaglebioproducts.com
(toxin testing)

Attn: Stephanie A. Smith, PhD
phone: (614) 519-0154 (cell)
www.beaglebioproducts.com

CAHFS Toxicology Laboratory
University of California, School of Veterinary Medicine
West Health Sciences Drive, Davis, CA 95616
(toxin testing)

Attn: Birgit Puschner
phone: (530) 752-6322
fax: (530) 752-3361
bpuschner@ucdavis.edu

CH2M HILL Applied Science Laboratory
1000 NE Circle Blvd., Suite 10350, Corvallis, OR 97330
(toxin testing - Microcystin)

Attn: Lab Customer Service Support
phone: (541) 768-3120 fax: (541) 766-2852
asl@ch2m.com

EcoAnalysts, Inc.
1420 S. Blaine St., Suite 14, Moscow, ID 83843
(toxin testing, identification and enumeration)

phone: (208) 882-2588
fax: (208) 883-4288
eco@ecoanalysts.com

GreenWater Laboratories/Cyano Lab
205 Zeagler Drive, Suite 302, Palatka, Florida 32177
(toxin testing, identification and enumeration)

phone: (386) 328-0882
fax: (386) 328-9646,
markaubel@greenwaterlab.com

King County Environmental Laboratory
322 West Ewing Street, Seattle WA 98119
(identification, enumeration, toxin testing)

Attn: Fran Sweeney
phone: (206) 684-2358
Francis.sweeney@kingcounty.gov

LSSU Environmental Analysis Laboratory
Lake Superior State University
650 W. Easterday Avenue, Sault Ste. Marie, MI 49783
(identification, enumeration, toxin testing)

Attn: Ben Southwell
phone: (906) 635-2076
bsouthwell@lssu.edu

PhycoTech
620 Broad Street, Suite 100, St. Joseph, MI 49085
(identification and enumeration)

phone: (269) 983-3654
fax: (866) 728-5579
info@phycotech.com

WATER Environmental Services, Inc.
9515 Windsong Loop NE, Bainbridge Island, WA 98110
(identification and enumeration)

Attn: Maribeth Gibbons, Pres.
phone: (206) 842-9382
mvg.water@gmail.com